STATE OF ALASKA

TONY KNOWLES, GOVERNOR

DEPARTMENT OF FISH AND GAME

DIVISION OF WILDLIFE CONSERVATION

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Ann Terbush
Chief, Permits Division
Office of Protected Resources
Marine Mammal Permits Division
1315 East-West Hwy., Rm 13126
Silver Spring, MD 20910

May 21, 2001

RE: Amendment 2 of Marine Mammal Permit No. 358-1564-01

Dear Ann,

This letter requests permission to amend Marine Mammal Permit No. 358-1564-01 which authorizes the take of Steller sea lions (*Eumetopias jubatus*) and harbor seals (*Phoca vitulina*) during scientific research of Steller sea lions in Alaska.

I am submitting this amendment as requested during the conference call of April 30, 2001 between your office, representatives of the Marine Mammal Commission and the Alaska Dept. of Fish and Game. This request is subsequent to Amendment 1, authorized on May 2, 2001.

I am requesting permission to amend the permit as follows:

1. Amend the frequency of Task 1a to allow annual surveys.

2. Amend the frequency of Task 1b (inadvertent harassment) the same as Task 1a.

3. Amend Task 3b as follows:
   - Include blubber biopsy
   - Include stomach tube as an alternative to enemas,

4. Amend task 4a as follows:
   - Increase the volume of blood drawn to120cc.
   - include muscle biopsy under the options for biopsy.
   - allow up to 4 takes/animal/year to account for the possibility of recaptures.

Recd
5/25/01

- Include injections of Evans Blue dye (this would come after the deuterium oxide in Task 4a).
- Include stomach tube as an alternative to enemas.

5. Amend task 4b to increase the number of SLTDR tags and VHF transmitters to 65/ 300. This would not increase the number of animals handled or the handling time. The increase in number of transmitters is to allow collaborative work recently funded by NMFS under the Steller Sea Lion Research Initiative to the University of Alaska, Anchorage.

6. Add Jamie King and Lorrie Rea to the Co-investigators list. These individuals were already added in Dec. 2000 (see enclosure) but their names do not appear on the most recent amendment.

7. Add the following people to the Research Assistants: Kimberlee Beckman, Greg Cambell, Raychelle Daniel, Robert DeLong, Frances Gulland, Heather Harmon, Bruce Heath, Marty Helina, Bob Gisner, Heather Ireland, Gary Snyder and Vicki Vanek,

Justification for these amendments are included in the enclosed material. Additionally, I have included a new copy of Table A with these amendments included in bold type.

We are hoping that these amendments can be added prior to our upcoming field trip which departs Anchorage on 11 June, 2001. The actual field work will begin on June 23, 2001. A hard copy of this application is in the mail. Please contact me if you have any questions concerning these amendments.

Sincerely,

Thomas S. Gelatt, Ph.D
Principal Investigator
Steller Sea Lion Program
907 267-2188
Fax: 907 267-2859

Justification for amendments to Marine Mammal permit No. 358-1564-01.

Task1a&b.

Aerial Survey – Increasing frequency of annual aerial surveys. Previously, aerial surveys of Steller sea lion haul outs have been conducted every other year. This was a logistical and financial decision more than a scientific one. We received an increase in funding for the 2001 fiscal year and anticipate similar increases in the future. This now allows surveys to be flown annually. In addition, a recently developed method of aerial surveying using medium-format cameras has shows promise to be more effective in identifying ageclasses from photos than traditional 35mm oblique photos. Using medium format, color aerial photography to survey sea lion numbers has been shown to provide estimates with similar precision to drive counts but without disrupting the rookery (Snyder et al. 2001).

Task 3b.

Blubber biopsy - The use of blubber for fatty acid analysis was described in the original application for this permit on 20 January, 2001. Fatty acid analyses from blubber samples is being used to investigate the process of nutritional independence in juvenile Steller sea lions. The original permit did not include blubber biopsies from young pups yet these individuals are known to be gaining all of their nutrients from milk. Samples from nursing pups are necessary to collect baseline values of the standard output for a pup/juvenile that is still nursing.

Stomach tube – The current permit allows for enemas to collect dietary prey items from the digestive tract. However, the rate of prey item assimilation within the GI tract is not consistent and some items become too degraded during digestion to provide useable data. Using a stomach tube while the animal is under anesthesia is a far more productive method for obtaining dietary samples, can provide recent dietary information and above all is quicker and easier to perform and less disturbing to the animal than enemas.

Task 4a.

Blood sampling – An increase in the amount of blood collected and the inclusion of Evans Blue dye are requested to provide samples for research examining blood oxygen stores. The size of blood oxygen stores will be determined by using the Evan's blue methodology to estimate plasma volume (ICSH 1973; Foldager and Blomqvist 1991) and field measurements of hematocrit and hemoglobin to determine total blood volume and oxygen carrying content (Kooyman et al. 1980; Ponganis et al. 1993; Costa et al. 1998). To determine total blood volume, measurements will be made using standard techniques (commonly used in measurement of total blood volume in humans and in other pinnipeds, see Costa *et al*. 1998). A 7-ml blood

sample will be drawn from either interdigital hind flipper vein. We will then give the animal an intravenous injection of 3-5 cc Evans Blue dye (0.5 mg/kg dose) into the same vessel. After the injection, but before the needle is removed, blood will be drawn into the syringe and used to flush out the needle. This ensures that the injection was intravenous and that all of the dye has been injected. We will take four more blood samples at 5-7 minute intervals. Each blood sample will constitute 7 ml, for a total of 35 ml drawn from each animal. These samples cannot be taken from the whole blood collections used in the health and condition studies identified in the original application and permit. The total time for this procedure is no more than 1 hour, and will be conducted within the 2 hour equilibration period needed for the deuterium dilution technique to assess body composition currently permitted

In order to address questions about the foraging strategies used by Steller sea lions, it is essential that the aerobic dive capacity of the seals be estimated. This will allow us to determine whether long foraging dives are anaerobic in nature, and permit a more accurate modeling of dive cycles (Kooyman et al. 1980; Ydenberg and Clark 1989; Fedak and Thompson 1993). To estimate the aerobic diving capacity of sea lions we will measure blood and muscle oxygen reserves, and combine these values with estimates of body composition, muscle mass and lung volume (based on published allometric equations) in order to arrive at an estimate of total body oxygen reserves. The ratio of total body oxygen reserves to resting metabolic rate has been shown to be a reasonable predictor of the aerobic dive limit in adult seals (Kooyman 1989). To determine the blood oxygen stores we will use the Evan's blue method in combination with field measurements of hematocrit and hemoglobin (Costa et al. 1998). Muscle oxygen stores will be determined by collecting a small tissue biopsy (<0.5g) and analyzing that muscle sample for myoglobin content (Reynafarje 1963; Kanatous 1997; Kohin 1998). In order to estimate muscle mass we will determine the body condition of seals from both ultrasonic measurements of blubber depth and estimates of total body water (Costa 1987; Bowen and Iverson 1998).

Muscle Biopsies - A small muscle biopsy will be taken to analyze myoglobin content and fiber type. The following procedure has been performed on a number of different pinniped species without adverse effects or complications (Kanatous 1997; Reed, Butler et al. Ponganis et al. 1993). Prior to sampling, we will inject a local anesthetic of 2-ml Xylocaine subcutaneously and intramuscularly at the sampling site if the animal is not under general anesthesia (isoflourane gas). The sampling site will be cleaned with a Betadine scrub and we will make a small incision with a scalpel blade. A closed muscle biopsy canula needle of 5 mm will be inserted into the incision and pushed through the fascia into the muscle layer. Once the needle is about 1 cm deep into the muscle, we will open the needle and apply pressure to force muscle into the needle. The needle is then closed and withdrawn and pressure is applied to the wound. In this manner, we will collect two samples (25-35 mg of muscle each) from the same site (at different angles). Two samples are necessary for the myoglobin assay requires flash frozen tissues, and the fiber type assays require fixed tissue. We

will irrigate the biopsy site with Betadine but no sutures are needed for the wound. The biopsy needles will be sterilized with a liquid cold sterilizer between animals. To reduce the number of incisions, the muscle biopsy will be collected from the same site a the blubber biopsy currently permitted.

Include up to 4 takes/animal/year - Longitudinal studies of animal health and condition rely on the ability to re-sample individuals over time. Young Steller sea lions are known to repeatedly visit divers during underwater captures despite being previously captured. With the increased number of marked individuals in the population, the potential for resampling is also increasing. Our underwater dive capture techniques have improved over time to the point where an individual sea lion can now be targeted. Additionally, because we are conducting capture work throughout Southeast Alaska and juveniles tend to distribute among different haul outs, the potential for recapturing an individual marked at a different rookery now allows a unique method of studying the timing of weaning, one of the great unknowns in Steller sea lions.

Task 4b.

Increase the number of satellite-linked time-depth recorders to 65 - The Steller Sea Lion Research Initiative recently funded Dr. Jennifer Burns from the University of Alaska, Anchorage to investigate foraging behavior and movement patterns of Steller sea lions using high-resolution satellite relay data loggers. This project will meet part of the requirements for a Master of Science degree for Michael Rehberg. Michael is a biologist in the ADF&G sea lion program and will be deploying these instruments during our planned capture trips. This amendment does not request to increase the number of animals handled or the handling time. The animals instrumented will continue to be a subset of the 300 animals permitted to handle and anesthetize.

Research Conditions

Amend the Co-investigators list – Co-investigators Lorrie Rea and Jamie King were added to this permit in January, 2001 but their names were omitted from the last permit amendment.

Amend the Research Assistants list - The capture and handling of Steller sea lions requires a large number of people for safe and efficient processing. The Steller sea lion program of ADF&G does not have enough people to adequately handle all of the work in a timely manner. For this reason we often obtain assistance from other

biologists and graduate students. In addition anesthesiologists and veterinarians are often contracted to provide immobilization and pathology assistance. I have listed these individuals under the heading Research Assistants. All Research Assistants will be conducting work under the direct supervision of the Principal Investigator or Co-investigators.

ICSH. 1973. A report by the international committee for standardization in haematology. Standard techniques for the measurement of red-cell and plasma volume. Brit.J.Haem. **25**: 801-814.

Foldager, N., and Blomqvist, C.G. 1991. Repeated plasma volume determination with the Evans blue dye dilution technique: the method and the computer program. Comput.Biol.Med. **21**: 35-41.

Kanatous, S. 1997. Muscle myoglobin levels and mitochondrial densities in pinniped muscles. Ph.D. Thesis. Texas A &M University, Galveston.

Ponganis, P.J., Kooyman, G.L., and Castellini, M.A. 1993. Determinants of the aerobic dive limit of Weddell seals: analysis of diving metabolic rates, postdive end tidal PO_2's, and blood and muscle oxygen stores. Physiol.Zool. **66**: 732-749.

Snyder, G. M., K. W. Pitcher, W. L. Perryman, and M. S. Lynn. 2001. Counting Steller sea lion pups in Alaska: An evaluation of medium-format, color aerial photography. Marine Mammal Science **17**:136-146.

Reilly, J.J., and Fedak, M.A. 1990. Measurement of body composition of living grey seals by hydrogen isotope dilution. J.Appl.Physiol. **69**: 885-891.

Table A. Annual takes during Steller Sea Lion scientific research. SSL=Steller sea lion, HS=harbor seal.

Task No.	**Type of Take (Activities)**	**Spp./ Age class**	**# Animals**	**# Takes/ animal/ year**	**Season/ Frequency**	**Location**
Task 1. 1.a	Aerial surveys photograph non-pups Breeding season	 SSL Non-pup Pups	45,000 15,000	 1	June-July. **annually**	Southeast Alaska
1.b	Inadvertent harassment during surveys	HS All ages	5,000	1	June-July **annually**	Gulf of AK Aleutian Isl.
Task 2. 2.	Land-based pup counts ≤ 10 selected rookeries	SSL non-pups pups	15,000 10,000	1	June-July annually	Southeast AK, Western Aleutian Isls.
Task 3. 3.a[1]	Capture during breeding season. weigh, measure, flipper tag [retain flipper punch], relase, recapture, weigh, blood sample, release, recapture, weight blood sample, if possible.	SSL pups	700[1]	2	June-July	Alaska-wide [40+ rookeries]
3.b.[2]	Specimen collection: blood [≤25cc], skin, hair, vibrissae & nail samples; fecal loop or rectal swab, **blubber biopsy**, swabs of dermal lesions and ocular, rectal/vaginal; enemas **or stomach tube** pups >1/2 mos;	SSL pups	350[2]/ 700	2	June-July	Alaska-wide [≤25 rookeries]

Task No.	Type of Take (Activities)	Spp./ Age Class	# Animals	# Takes/ animal/ year	Season/ Frequency	Location
3.c[3]	Branding pups <1 1/2; anesthetize w/isoflurane. Equal number of male/female branded	SSL pups	600[3]/ 700	2	June – July (2001, 2002, 2003, 2004)	Alaska-wide [40+rookeries]
Task 4. 4.a	Capture pups > 2 mo and juveniles hoop net and underwater rope lasso; gas anesthesia; weigh, measure, flipper tag [retain flipper punch]; brand Specimen collection: blood [**120cc**], tissue sample, **muscle biopsy**, fecal loop or rectal swab, swabs of dermal lesions and ocular, rectal/ vaginal; tooth extraction; Enemas or **stomach tube** Biopsy, skin and blubber, [use lidocaine]; deuterium oxide, **Evans Blue dye**	SSL pups>2 mos/ juveniles 1 to 3 yrs	300	**4**	All year	Alaska-wide [40+ rookeries]
4.b.	SLTDR TAGS and VHF transmitters	SSL pups/juv >1.5 mos	**65**[4]/300	2	All year	Alaska-wide [40+rookeries]
Task 5. 5.	Capture any age/sex class: blood [70cc]; fecal loop or rectal swab, swabs of dermal lesions and ocular, rectal/vaginal; vibrissae; tooth; Enemas; Biopsy, blubber	SSL All ages	10	2	All year	Alaska-wide [40+rookeries]

Task No.	Type of Take (Activities)	Spp./ Age Class	# Animals	# Takes/ animal/ year	Season/ Frequency	Location
Task 6. 6.	Incidental Disturbance during scat collection, capture operations, observational activities, remote sensing station installation and maintenance	SSL All ages	7,000	1	All year	Alaska-wide [40+rookeries]
Task 7. 7.	Accidental Mortality during field operations	SSL All ages	5	1	All year	Alaska-wide [40+rookeries]

[1]Subset of pups in Task 2.; [2]Subset of pups in 3.a.; [3] Subset of pups in 3.a.; [4]Subset of animals in 4.a

B. Research Conditions

1. The following individuals may participate in the conduct of the research authorized herein as Co-investigators: Dr. Don Calkins, Kim Raum-Suryan, Mike Rehberg, Kelly Hastings, Grey Pendleton, Walt Cunningham, Dennis McAllister, Kathy Burek, William Taylor, and Chris Curgus, Ken Pitcher, Jennifer Burns, Millie Gray, **Lorrie Rea and Jamie King**; Julie Richmond, Matthew Myers**, Kimberlee Beckman, Greg Cambell, Raychelle Daniel, Robert DeLong, Frances Gulland, Heather Harmon, Bruce Heath, Marty Helina, Bob Gisner, Heather Ireland, Gary Snyder and Vicki Vanek** as Research Assistants.

STATE OF ALASKA

TONY KNOWLES, GOVERNOR

DEPARTMENT OF FISH AND GAME

DIVISION OF WILDLIFE CONSERVATION

333 Raspberry Road
Anchorage, AK 99518-1599
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18 December, 2001

To: Ann Terbush

From: Tom Gelatt

Re: Reallocation of takes under the ADF&G Steller sea lion research permit No. 358-1564-00

The Alaska Department of Fish & Game (ADF&G), Steller sea lion research program requests reallocation of takes under Marine Mammal Permit No. 358-1564-00, as per recent discussions between Ann Terbush, Ruth Johnson, Tammy Adams and Tom Gelatt in Vancouver, B.C. on Dec. 8, 2001. Specifically, the ADF&G requests an annual reduction of 5 capture takes for pup/juvenile Steller sea lions and in increase of 5 accidental mortalities.

Permit 358-1564-00, Task 4a. currently authorizes **300** capture takes annually for pups > 2 months of age and juveniles. These animals are captured using a hoop net or underwater rope lasso, immobilized using gas anesthesia and sampled using various protocols explained previously. Permit 358-1564-00 also currently authorizes the annual accidental mortality of **5** Steller sea lions of any age. We are requesting to reduce our maximal annual capture of pups and juveniles in Task 4a to 295, and to increase our accidental mortality of sea lions of any age to **10**.

This request does not imply that we anticipate higher rates of mortality for captured animals. Rather, the intensity and scope or our research has increased recently especially in regard to pup handling. Additionally, one of the leading hypotheses for the sea lion decline is the idea of a nutritional stress leading to starvation. An investigation of this hypothesis requires the handling of diseased or moribund animals when possible. These animals by nature of their condition are at high risk of mortality, however to avoid handling them because of their condition would negate the focus of the research and risk overlooking a possible cause of the decline. As in the past and as we have assured personnel in your office, the first priority of the ADF&G and our co-investigators is minimize the likelihood of injury or accidental death of any sea lions during our field investigations.

Please do not hesitate to contact me if you require additional information to address this request.

Sincerely,

Thomas S. Gelatt, Ph.D.
Steller Sea Lion Program Leader

STATE OF ALASKA

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Dr. Tammy Adams
Permits Division
United States Department of Commerce
National Oceanic and Atmospheric Administration
National Marine Fisheries Service
Silver Spring, Maryland 20910

MAR - 5 2002

February 21, 2002

Re: Amendment to Marine Mammal Permit No. 358-1564

Dear Tammy,

This letter requests an amendment to Permit No. 358-1564-01, authorization to take Steller sea lions in Alaska. Three procedures are outlined; 1. the use of Bioelectric impedance analysis has been permitted under previous Principal Investigators working on Steller sea lions and is requested to allow necessary comparisons between stocks, 2. the attachment of VHF transmitters to pups and juveniles will allow researchers to follow animals when out of visual range, and 3. the muscle biopsy procedure is intended to supplement the amendment requested in May, 2001. Please note that requests no's. 1 and 2 are new while request no. 3 adds to the May, 2001 amendment (No. 2) request and simply provides additional information and clarity to supplement what was outlined in the DRAFT EA regarding muscle biopsies and stipulates that two sites would be sampled. Additionally, I am including an attachment providing some additional citations of the physiological procedures for your reference.

The procedures outlined here do not increase the number of takes currently permitted because they all occur on animals handled for other permitted activities, but do increase the number of procedures carried out on an anesthetized animal. Therefore, it is my hope that you can easily incorporate these requests into the present EA and BiOp. If you are unable to incorporate this amendment or, if in your opinion the addition of this amendment would greatly impede the progress of the EA or BiOp, please contact me and we will discuss withdrawing the request.

1. **Bioelectric Impedance Analysis (BIA)** - Amend task 4a. to include BIA on up to 300 sea lions greater than 2 months of age. This procedure has been performed before under different permits and is being requested in order to allow reliable comparisons between animals sampled in the eastern stock by ADF&G and work being carried out by the National Marine Mammal Lab. As noted in the draft EA, this procedure is performed on anesthetized animals and requires the insertion of four 1.5" 20-guage needles subcutaneously behind the skull and near the tail. The needles are attached to electrodes and a small current is sent from one set of needles to the other.

The rate of current is measured and used as a correlate to other condition indices. The rate of current is proportional to the amount of fat in the body and thus BIA is used as a tool to measure body condition. The sea lions do not show any apparent response to the procedure.

2. **VHF transmitters** -

A. Amend task 4.b. by including another subheading for 130 VHF-tags only for pups and juveniles of any age. This procedure is requested for projects already underway. Permit 358-1564-01 currently allows the attachment of up to 45 SLTDR and VHF tags and an increase has been requested to 65. However, those are tags with both transmitters incorporated into the same instrument for purposes of local tracking and instrument recovery. This amendment requests permission to attach much smaller (50 g, 30 x 60 x 10mm) transmitters to 100 pups for the purpose of local tracking. The pups will have the radios attached at the time of branding (within task 3.c.) and will yield information on pup mortality during the post-branding period for a study of the effects of branding on pup survival. Field observers will track the animals on a daily basis, remote data loggers will be set up on outlying islands to track emigration and aerial surveys will be used to search for animals away from land stations.

B. During our juvenile capture operations, not all captured animals are instrumented with SLTDR and VHF packages because of the cost of the combined instruments. However, in southeast Alaska other Steller sea lion researchers (NMFS, Auke Bay Lab, M. Sigler) have requested the opportunity to track sea lions during their sea lion-fishery studies. By tracking from the air and ship, the NMFS researchers will be able to conduct fishery surveys in the areas that known sea lions are foraging, a much more precise sampling design than the current method of watching for any sea lions and then sampling. VHF transmitters are smaller, cheaper and easier to apply than the combined instruments. This amendment requests to attach up to 30 VHF transmitters to sea lions captured for physiological sampling but not insrumented with the combined SLTDR-VHF packages. As with other instrument attachments, marine epoxy will be used to glue the instruments to the fur on the shoulder or head of an anesthetized sea lion and will drop off during the annual molt.

3. **Muscle Biopsies** – Clarify the requested amendment of task 4a requested in May 2001 to allow the procedure at two sites on the animal. A small muscle biopsy will be taken to analyze myoglobin content and fiber type composition. The following procedure has been performed on a number of different pinniped species without adverse effects or complications (Kanatous 1997; Reed et al. 1994; Ponganis et al. 1993). Prior to sampling, we will inject a local anesthetic of 2-ml Xylocaine subcutaneously and intramuscularly at the sampling site if the animal is not under general anesthesia (isoflourane gas). The sampling site will be cleaned with a Betadine scrub and we will make a small incision with a scalpel blade. A closed muscle biopsy canula needle of 5 mm will be inserted into the incision and pushed through the fascia into the muscle layer. Once the needle is about 1 cm deep into the muscle we will open the needle and apply pressure to position the muscle inside the needle. The needle is then closed, withdrawn and pressure will be applied to the site. In this manner, we will collect two samples (25-35 mg of muscle each). Since myoglobin concentrations vary between muscle groups, two samples are required to determine total myoglobin load (Dolar et al. 1999; Kanatous 1997; Reed et al. 1994). One sample will be taken from a muscle of high myoglobin concentration (pectoralis muscle group) and one from a muscle of low concentration (hind limb complex, same site as the blubber biopsy to minimize incisions).

We will irrigate the biopsy site with Betadine but no sutures will be needed for the wound. The biopsy needles will be sterilized with a liquid cold sterilizer between animals.

Once again, I stress that these amendments are intended to be included in the current revision of the EA rather than initiating another EA. I realize that this revision is likely becoming more and more confusing and I appreciate your diligence in tackling what appears to be an enormous task. If any part of this request is unclear or would present a problem please do not hesitate to contact me for additional information.

Sincerely,

Thomas S. Gelatt
Principal Investigator
Steller Sea Lion Program

References for muscle myoglobin.

Kanatous thesis already referenced.

Dolar, M.L.L., P. Suarez, et al. (1999). "Myoglobin in pelagic small cetaceans." The Journal of Experimental Biology 202: 227-236.

Reed, J. Z., P. J. Butler, et al. (1994). "The metabolic characteristics of the locomotory muscles of grey seals (*Halichoerus grypus*) harbour seals (*Phoca vitulina*) and antarctic fur seals (*Arctocephalus gazella*)." Journal of Experimental Biology **194**: 33-46.

References for BIA

Arnould, J. P. Y. (1995). "Indices of body condition and body composition in female Antarctic fur seals (*Arctocephalus gazella*)." Marine Mammal Science **11**(3): 301-313.

Gales, R., D. Renouf, et al. (1994). "Use of bioelectrical impedance analysis to assess body composition of seals." Marine Mammal Science **10**(1): 1-12.

Bowen, W. D., C. A. Beck, et al. (1999). "Bioelectrical impedance analysis as a means of estimating total body water in grey seals." Canadian Journal of Zoology **77**(3): 418-422.